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LABORATORY INVESTIGATION

A Custom Radiopaque Thermoresponsive Chemotherapy-Loaded Hydrogel for Intratumoural Injection: An In Vitro and Ex Vivo Assessment of Imaging Characteristics and Material Properties

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Abstract

Purpose Thermoresponsive hydrogels are gels which have different properties at varying temperatures. The objective of this study was to assess the material characteristics, imaging properties and chemotherapeutic drug release profile of a novel radiopaque thermoresponsive hydrogel in vitro, which is liquid at room temperature but solidifies at body temperature, to determine potential suitability for intratumoural delivery.

Materials and Methods An iodinated radiopaque thermoresponsive hydrogel was formulated using iodixanol at a range of concentrations and assessed for sol–gel transition, radiopacity and imaging using CT and US. A lead formulation containing iodixanol at a concentration of 9.22% weight by weight (w/w, g of iodixanol per g of hydrogel) was evaluated in vitro for injectability, disintegration and dual drug release of cisplatin and paclitaxel from the hydrogel formulation.

Results Radiopacity of the hydrogel increased in a concentration-dependent manner, but the highest concentration of iodixanol evaluated in this study (13.83% w/w)

adversely affected the sol–gel transition of the hydrogel; therefore, 9.22% w/w iodixanol hydrogel was identified as the lead formulation. This formulation was readily visible on both CT and US. The formulation was hand injectable through a range of clinically relevant devices, had a sustained disintegration profile for up to 28 days and was able to deliver a sustained release of chemotherapeutic drug for up to 10 days.

Conclusions Favourable in vitro and ex vivo imaging and material characteristics of this thermoresponsive gel are demonstrated, suggesting potential interventional oncology applications for image-guided intratumoural delivery of sustained-release chemotherapy.

Keywords Thermoresponsive hydrogel · Intratumoural injection · Radiopaque · Chemotherapy

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Introduction

Systemic chemotherapy, in conjunction with radiation and surgery, has long been the mainstay of cancer treatment. Lack of chemotherapy specificity, toxicity, off-target side effects and poor penetration into the tumour mass due to the altered tumour microenvironment limits efficacy, which has encouraged alternative delivery methods to be investigated [1–4]. One such approach is direct injection of chemotherapeutics into the tumour, delivering chemotherapeutics or other ablative agents directly to achieve high local concentrations while reducing systemic toxicity. In vivo animal studies have shown that direct intratumoural

instillation of a chemotherapeutic solution can deliver more than 10–30 times intravenous doses, with minimal side effects [5]. Intratumoural chemotherapy has also been explored clinically, with promising safety and proof of concept data generated [6–8]. Direct intratumoural instillation is, however, limited by rapid solution clearance from the tumour site, resulting in inaccurate and unpredictable dosing. Therefore, attention has turned to the development of drug delivery systems which can facilitate delivery and retention at the site of action, and sustained intratumoural release of chemotherapeutics [4].

Thermoresponsive hydrogels offer the possibility of an injectable liquid at room temperature that can phase transition to a semi-solid hydrogel at physiological temperatures and have been investigated in pre-clinical and clinical settings for intratumoural drug delivery in solid tumours [9, 10]. This is an attractive drug delivery platform, due to the potential for image-guided administration without the need for surgical implantation of hardware. In situ gelation permits hydrogels to mould to the target tissue, facilitating a better fit than preformed implants [11], and facilitates sustained release of the drug at the required site of action [12–14]. Hydrogels can also be disintegrable, and the rate of disintegration can be tailored without the need for surgical removal.

Only a handful of thermoresponsive hydrogels have progressed to clinical trials, with varying degrees of success [4, 15–19]. This lack of clinical translation may be due to the fact that whilst the studied gelling properties are of importance, other factors which are of clinical relevance such as visibility using image guidance, injectability, hydrogel distribution, drug release, retention and disintegration have not been given sufficient consideration during development. This study explores these clinically relevant factors of a novel thermoresponsive hydrogel formulation that has been specifically developed for intratumoural delivery.

Methods

Formulation of a Radiopaque Thermoresponsive Hydrogel With and Without Chemotherapeutic Drug Loading

A novel proprietary thermoresponsive hydrogel developed in a university research laboratory was prepared with a commercially available iodinated contrast agent iodixanol (Visipaque® 320 mg I/ml, GE Healthcare, IL, USA). Iodixanol was added at concentrations of 0% w/w, 4.61% w/w, 9.22% w/w or 13.83% w/w, based on previous work carried out by Fatimi [19]. A dual drug-loaded version of this radiopaque thermoresponsive hydrogel was prepared

using two widely used chemotherapeutic agents, cisplatin and paclitaxel. Cisplatin was loaded into the aqueous phase of the thermoresponsive hydrogel with stirring. Solubilisation of paclitaxel in a formulation excipient was undertaken prior to drug loading into the thermoresponsive hydrogel.

Assessment of Material Characteristics of Iodixanol-Labelled Hydrogel Formulation

The thermoresponsivity and shear thinning properties of the hydrogel formulations were assessed using oscillatory and flow measurements, performed on a constant stress rheometer (AR-1000, TA instruments, Delaware, USA). The rheometer was equipped with cone/plate geometry (40 mm diameter, 4° cone angle). Degassed samples were dispensed onto the temperature controlled rheometer plate, pre-equilibrated to 20 °C and covered with a solvent trap during testing. Oscillatory temperature sweeps from 20 to 40 °C were performed to determine sol–gel transition temperatures. The viscosity properties of the thermoresponsive hydrogel in response to an applied shear stress were also investigated in order to predict the material behaviour during injection. In response to an increasing shear stress applied shear thinning materials will decrease viscosity, while shear thickening materials will increase viscosity. Shear thinning materials are, therefore, the most suitable for injection purposes as the decreasing viscosity of the injectate will facilitate easier injection. Shear thinning properties of hydrogel formulations were assessed at 20 °C under increasing shear stress from 1 to 100 Pa. All experiments were repeated in triplicate. Data were processed using TA Data Analysis software.

Measurement of Radiopacity of Iodixanol-Labelled Hydrogel Formulation

2 ml of iodixanol-labelled hydrogel formulation was added per well in a 12-well plate. Radiopacity was determined using a commercially available computed tomography (CT) machine (Ingenuity Core 128, Philips Healthcare, Best, The Netherlands). Spiral volumetric acquisition through each 12-well plate was obtained using 0.8 mm slice thickness, 0.4 mm reconstruction interval, 168 mA s and 100 kV. A region of interest (10 mm diameter) was positioned centrally within each well on a coronal reformat for acquisition of density measurements, ensuring only hydrogel was included within the region of interest, excluding both air and plate. The average density was calculated (minimum, maximum and standard deviation also recorded). All measurements were carried out in triplicate. Data shown are represented as the mean ± standard error of the mean (SEM) ($n = 3$).

Assessment of Distribution of Iodixanol-Labelled Hydrogel in Ex Vivo Animal Tissue

Ex vivo tissue (calves liver) was used to assess the distribution of injected radiopaque thermoresponsive hydrogel. Prior to injection, the ex vivo tissue was heated to 37 °C in a water bath. Internal tissue temperature was recorded using a tissue thermometer. 5 ml of hydrogel with 9.22% w/w iodixanol was injected into the ex vivo tissue model using an 18 G 5 cm vascular access needle (Cook Medical, IN, USA) at a constant rate to assess distribution of injected thermoresponsive hydrogel in tissue. Three injections were made. Administration was monitored using a commercially available 12 MHz linear ultrasound (US) probe (Xario, Toshiba, Tokyo, Japan) in grayscale B-mode. Ex vivo distribution post-injection was imaged using CT as outlined previously.

Assessment of Injectability of Hydrogel Formulation

Uniaxial tensile testing was carried out to determine the force required to expel hydrogels from a syringe fitted with a needle or catheter, using a mechanical testing machine (Z050, Zwick/Roell, Ulm, Germany), fitted with a 5-kN load cell (Fig. 1). Samples were loaded into 2 ml Leurlok® syringes, and the required medical device was attached to the syringe via Luer-lok® (Table 1). A preload of 1 N was applied, and the end of test was determined to be the maximum extension (8.5 mm); the distance equivalent to 0.5 ml of hydrogel (measured using Vernier callipers). The speed of injection was defined as 2 ml/min, and the solution expelled from the catheter was collected in a vial. All measurements were carried out in a minimum of triplicate. Results are represented as the mean ± SEM (n = minimum of 3).

Disintegration Studies

1 g of the radiopaque thermoresponsive hydrogel formulation (blank or drug-loaded) containing 9.22% w/w iodixanol was added to a glass vial and submerged in a shaking water bath at 75 rpm, maintained at 37 °C, for 30 min to ensure complete gelation had taken place. 1 ml of pre-warmed phosphate-buffered saline (PBS) (pH 7.4) was added to the gel. At predetermined timepoints (4 h and day 1, 2, 3, 5, 7, 10, 14, 21 and 28), PBS was completely removed from the glass vial and the weight of the hydrogel + glass vial was recorded. After weighing, 1 ml of fresh pre-warmed PBS was added to the glass vial. All experiments were carried out in triplicate, for three independent batches. Results are represented as the mean of three independent experiments ± SEM (n = 3).

Chemotherapeutic Release Studies

1 g of drug-loaded radiopaque thermoresponsive hydrogel containing 9.22% w/w iodixanol was added to a glass vial and submerged in a shaking water bath at 75 rpm, maintained at 37 °C, for 30 min to ensure complete gelation had taken place. 1 ml of pre-warmed PBS (pH 7.4) was added

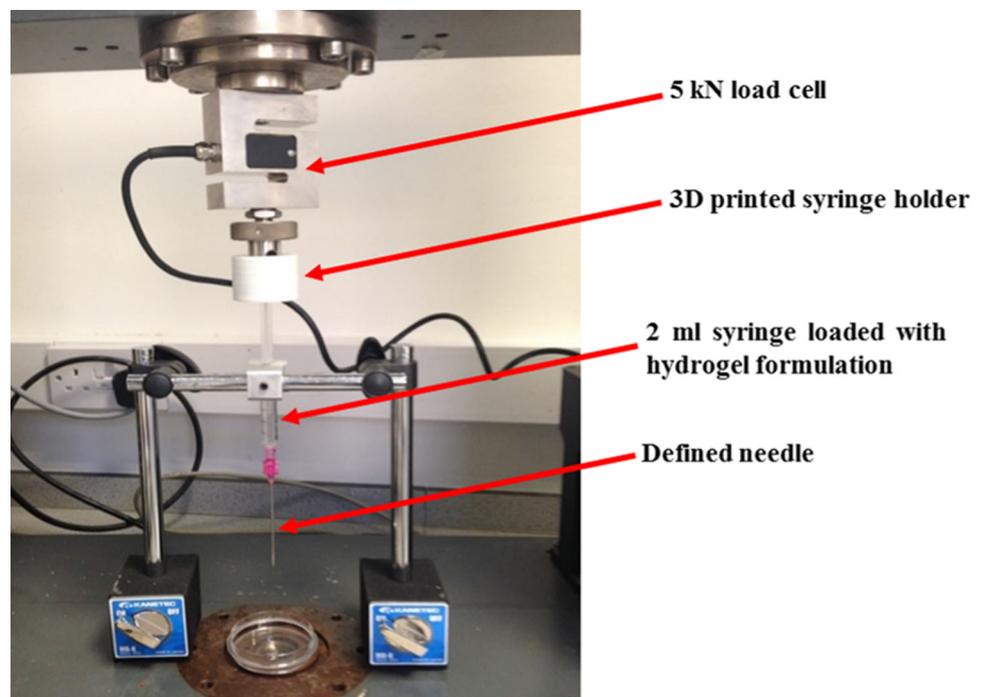Fig. 1 Representative image of injectability testing using a Zwick mechanical testing machine

5 kN load cell

3D printed syringe holder

2 ml syringe loaded with hydrogel formulation

Defined needle

Table 1 Summary of medical devices used in injectability testing of various thermoresponsive hydrogel formulations

Medical device	Needle gauge (G)/catheter diameter (Fr)	Length (cm)
Cook medical needle	18 G	7
Cook medical needle	18 G	20
Chiba biopsy needle	20 G	10
Terumo spinal needle	23 G	9
Cordis catheter	5 Fr	65

to the gelled hydrogel, and at predetermined timepoints (4 h and day 1, 2, 3, 5, 7, 10, 14, 21 and 28) PBS was completely removed from the glass vial and then replaced with 1 ml of fresh pre-warmed PBS. Samples were transferred to a centrifuge tube and stored at − 20 °C until analysis. Analysis of released paclitaxel was performed using liquid chromatography (Agilent Technologies 1120 Compact LC, CA, USA) with a UV detector, based on a method by Kraitzer et al. [20]. The mobile phase was water/acetonitrile (45:55), with a flow rate of 1 ml/min, a run-time of 10 min and detection at 227 nm. Analysis of released cisplatin was performed by quantifying platinum using inductively coupled plasma optical emission spectrometry (Liberty 150 ICP-MS, Varian Inc., CA, USA) at a detection wavelength of 214.423 nm, with a plasma flow of 15 L/min, auxiliary flow 1.5 L/min and nebulizer pressure of 200 kPa. All experiments were carried out in triplicate, for three independent batches. Results are represented as the mean of three independent experiments ± SEM ($n = 3$).

Results

Impact of Iodixanol on Sol–Gel Transition of Hydrogel Formulations

Figure 2a shows the effect of increasing concentrations of iodixanol on the material characteristics of the hydrogel. The storage modulus (G′) is the elastic component of the hydrogel, which is a measure of structure within the hydrogel. The sol–gel phase transition was marked by an abrupt increase in the G' of the gel, indicating a transition from liquid to semi-solid state at physiological temperatures as observed in Fig. 2b. Concentrations of iodixanol up to 9.22% w/w did not significantly impact on either the sol–gel transition temperature or the strength of the hydrogel. However, increasing the iodixanol concentration to 13.83% w/w caused a significant reduction in sol–gel transition temperature and an increase in the strength of the hydrogel.

Radiopacity of Iodixanol-Labelled Hydrogel Formulations

The addition of iodixanol solution to the hydrogel formulation was seen to produce a homogenous gelled formulation at all concentrations evaluated, as evidenced by CT imaging (Table 2). Addition of iodixanol solution resulted in increased radiopacity in a concentration-dependent manner. Gelation only minimally reduced radiopacity in all three iodixanol-labelled hydrogel groups. Due to favourable rheological and radiopacity characteristics, the hydrogel containing 9.22% w/w iodixanol was selected for further assessment.

Distribution of Iodixanol-Labelled Hydrogel in Ex Vivo Animal Tissue

Injection into ex vivo calves tissue (liver) demonstrated that the radiopaque thermoresponsive hydrogel (9.22% w/w iodixanol) was readily injectable, and moulded to the shape of the target tissue. On US, it was found to be clearly visualised, anechoic and without speckling (Fig. 3a). It demonstrated excellent radiopacity on CT achieving Hounsfield units far in excesses of liver parenchyma even with the lowest strength formulations (as outlined in Table 2). The hydrogel was seen to pool in parenchyma around the needle tip at commencement of injection, and as resistance (and presumably interstitial pressures) increased, the thermoresponsive hydrogel formulation subsequently began to spread along vascular and biliary channels before undergoing gelation. Following gelation, the thermoresponsive hydrogel moulded to the shape of the tissue, without further spread indicating successful transition from liquid to semi-solid had occurred (Fig. 3c–e).

Injectability of Hydrogel Formulations

Rheological measurement of viscosity at 20 °C demonstrated shear thinning, i.e. a decrease in viscosity with increasing shear, which is beneficial for injectability (Fig. 4a). Radiopaque hydrogel was injectable across all needle gauges evaluated, with only the 23 G device required a force greater than 50 N to achieve injection (Fig. 4b). Injection through a 65-cm 5-Fr catheter was also

Fig. 2 a Addition of iodixanol to the hydrogel formulation impacts the rheological properties of the thermoresponsive hydrogel in a concentration-dependant manner. Rheogram of the oscillatory temperature sweeps from 20 to 40 °C of hydrogel formulations containing different concentrations of iodixanol solution. **b** Representative image of hydrogel formulation at room temperature, in liquid state, and following incubation at 37 °C, in gelled state. Hydrogel contains colouring for clarity
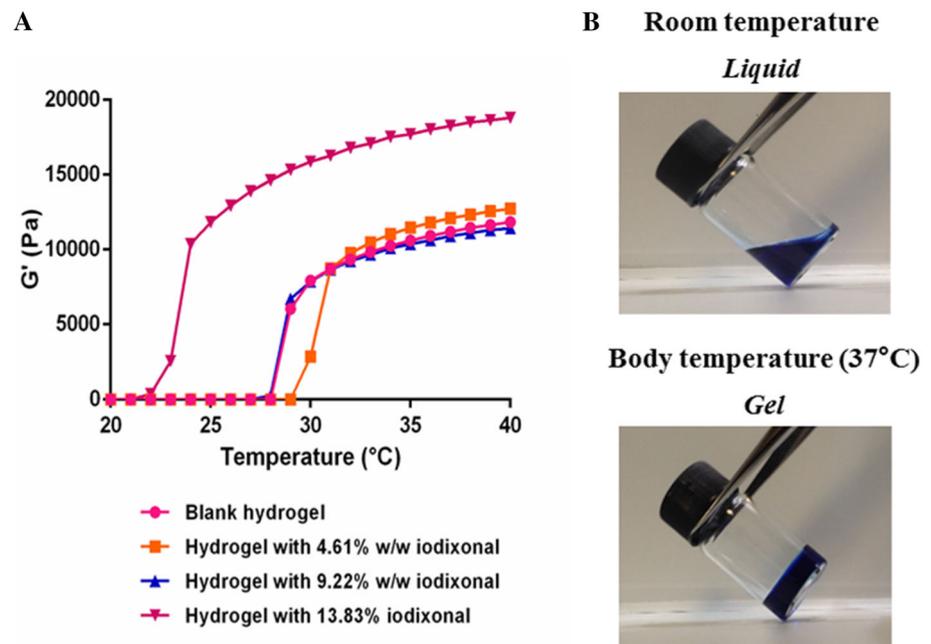
shown to be feasible. A force of 0–10 N was considered to correspond to an injection process which would be "easy to inject by hand", and injections which require forces up to 50 N to complete can be defined as "injectable by hand" [21].

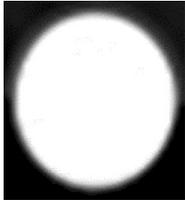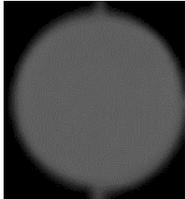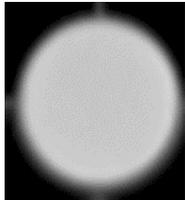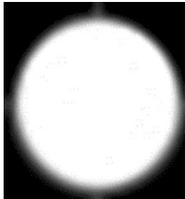## Disintegration and Chemotherapeutic Release from Hydrogel Formulations

Similar disintegration profiles for both the blank and drug-loaded radiopaque thermoresponsive hydrogel were observed in vitro over 28 days (Fig. 5a), with both formulations demonstrating extended disintegration profiles. An initial decrease in mass was observed for both formulations after 4 h of exposure with a subsequent steady decrease in mass observed thereafter until 168 h, after which mass loss levelled off, with approx. 20% of the hydrogel still present at day 28. It is hypothesised that cross-linking occurring between components of the gel after transitioning to a gel at 37 °C contributes to the extended disintegration profile of the gel. A slow disintegration of the hydrogel is important to ensure it remains in situ for a sufficient period of time to enable the chemotherapeutic drug load to be released at the required site of action. In vitro release study of a dual drug-loaded hydrogel showed sustained release of cisplatin and paclitaxel for up to 7 days (Fig. 5b). A significant burst release of both chemotherapeutic agents was observed in the first 24 h, with a higher burst and shorter sustained release of cisplatin. This is to be expected as cisplatin has significantly higher water solubility than paclitaxel and therefore

will diffuse more rapidly into the surrounding aqueous medium driven by simple diffusion. Paclitaxel, with lower water solubility, showed a smaller burst release and a more sustained release profile for up to 7 days from the hydrogel. The rate of drug release from hydrogels has been attributed to chemical, physical and biological interactions between the hydrogel matrix, the drugs loaded and surrounding tissue, including the rate of gel disintegration [22].

Discussion

Thermoresponsive hydrogels can support the delivery of a range of chemotherapeutic agents, achieving high, and lasting, local concentrations [3, 23–25]. To transition to such clinical use however, these hydrogels must be both easily visible on imaging, and hand injectable. This study demonstrates that combining an off-the-shelf iodinated contrast preparation with a novel thermoresponsive hydrogel formulation is feasible and leads to a clearly visible formulation both radiographically (on CT) and sonographically (on US). Furthermore, testing of viscosity and injectability demonstrates these formulations are injectable with forces that can be generated by hand. Preliminary in vitro disintegration studies suggest that the hydrogel has a prolonged disintegration profile, which would indicate the potential for prolonged retention within tissue. However, the effect of biological fluid may considerably alter the disintegration profile; therefore, this will require further in vivo evaluation. Assuming that prolonged retention is achievable this offers the potential for sustained

Table 2 Radiopacity of the thermoresponsive hydrogel formulations with increasing concentrations of iodixanol

	Liquid (HU)	Gelled (HU)	Coronal image of well plate on CT (window width 1488, level 346)
9.22% w/w iodixanol solution	1523.7 ± 7.09	N/A	
Thermoresponsive hydrogel with 0% w/w iodixanol	33.57 ± 2.34	22.4 ± 2.06	
Thermoresponsive hydrogel with 4.61% w/w iodixanol	900.03 ± 5.15	697.11 ± 19.28	
Thermoresponsive hydrogel with 9.22% w/w iodixanol	1666.23 ± 6.98	1586.0 ± 12.03	
Thermoresponsive hydrogel with 13.83% w/w iodixanol	2428.83 ± 19.01	2378.7 ± 13.9	
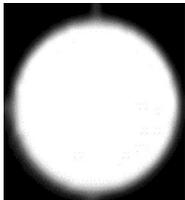
Data shown are representative of the mean ± SEM ($n = 3$)

HU Hounsfield units, *w/w* weight by weight

drug release, as drug release from a 3D matrix is a function of diffusion and erosion. These results suggest that the inherent properties of this thermoresponsive hydrogel offer potential for transition to the interventional radiology suite and future treatment opportunities. This is particularly promising for infiltrative tumours which can be accessed radiologically and demonstrate response to chemotherapy, but are not amenable to ablation or resection due to adjacent vital structures. Potential applications would therefore include locally advanced pancreatic cancer, cholangiocarcinoma, glioblastoma, and infiltrating mediastinal or peritoneal tumours.

The primary limitation of this study is its experimental nature. While demonstrating that contrast-labelled chemotherapy-labelled hydrogel can be injected under image guidance in ex vivo animal liver, translation into in vivo experimentation and clinical usage still requires much investigative work. In terms of clinical translation, further study is required to establish how thermoresponsive hydrogels behave on delivery into a variety of tissue types
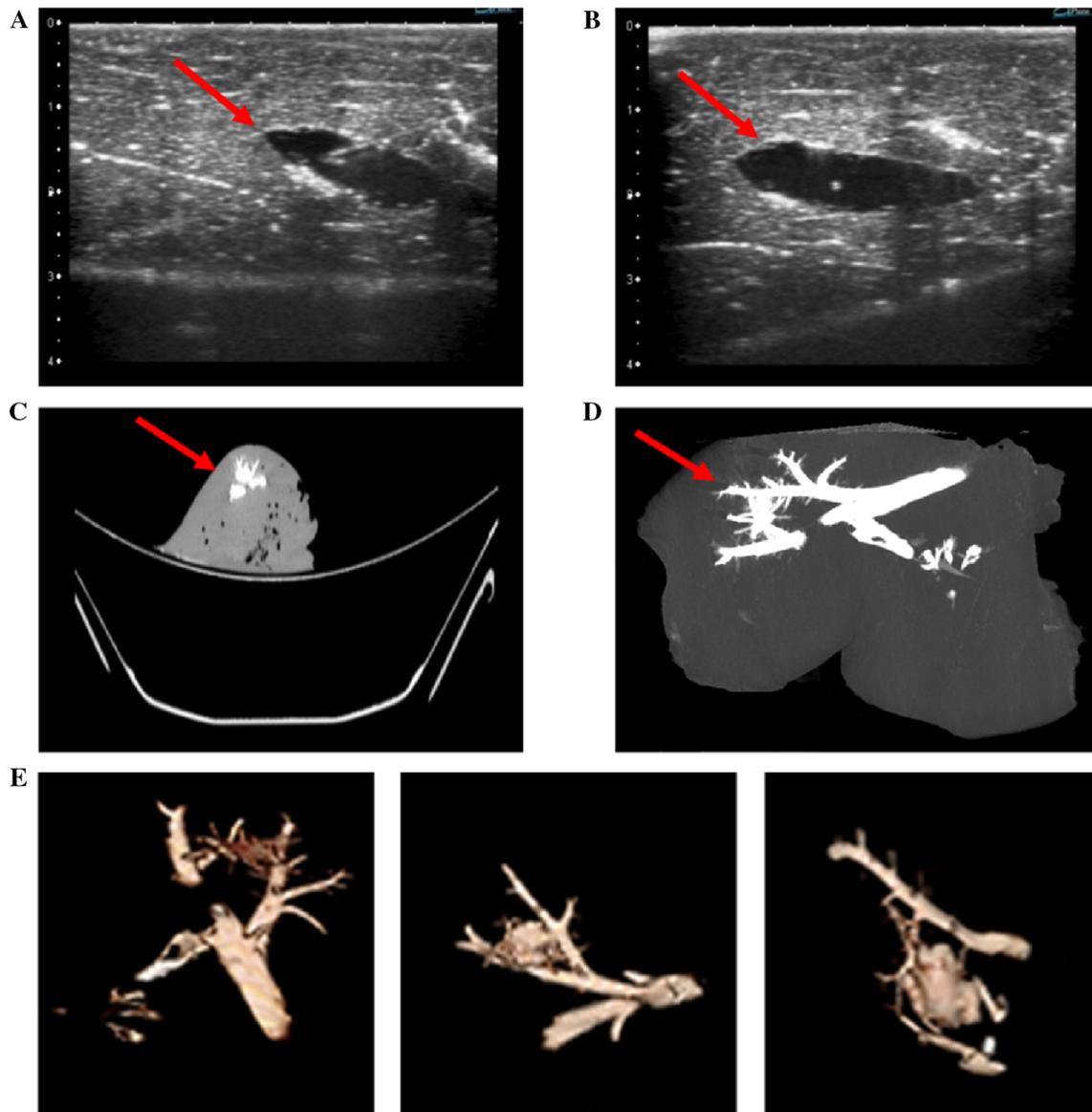
Fig. 3 Ex vivo tissue administration of the radiopaque thermoresponsive hydrogel can be visualised using CT and US. Representative a, **b** US and **c**, **d** CT images of radiopaque hydrogel distribution in ex vivo tissue and **e** CT images of radiopaque hydrogel pooling at parenchymal injection site (arrow), following which it spreads through vascular and biliary channels

and tumour types, in particular their in vivo disintegration profile. It will also be important to establish any toxicity associated with the use of the hydrogel. Work is currently ongoing in a number of pre-clinical xenograft cancer models to establish efficacy of both the blank and chemotherapeutic-loaded thermoresponsive hydrogel, and to determine whether there is any local or systemic toxicity associated with the hydrogel. The interventional radiologist will also require an understanding of how these hydrogels behave in soft tissue. In practice, injection force and spread within various tumours and organs (and even organs in various states such as cirrhosis) can feel different. In this study, the investigators note that within calf liver, the hydrogel pooled at the needle tip following hand injection, and flowed into vascular and biliary channels as injection pressure and volume increased. This would need to be validated in human tissue and for each clinical indication.

Conclusion

Thermoresponsive hydrogels can be designed to transition from an injectable liquid at room temperature, to a semi-solid at body temperature. This study demonstrates that this proprietary hydrogel can be loaded with various concentrations of iodinated contrast and chemotherapeutic agents
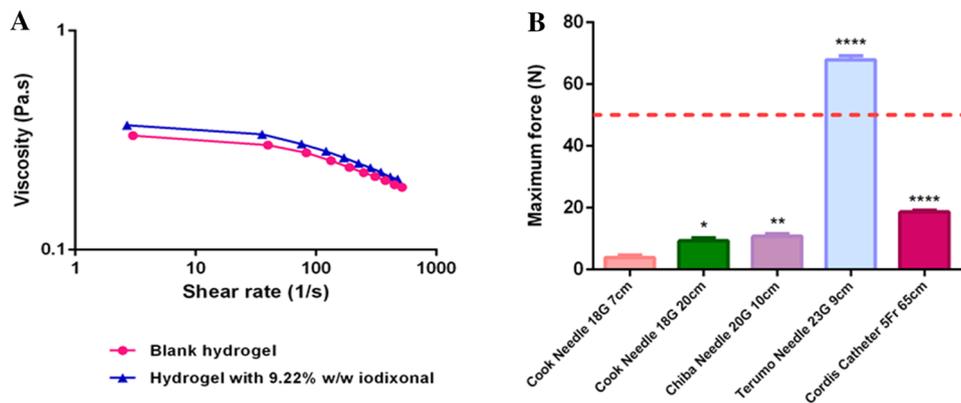
Fig. 4 **a** Unlabelled and contrast-labelled thermoresponsive hydrogel underwent shear thinning (a decrease in viscosity in response to increasing shear rate). Data shown is representative of the norm. **b** Injectability of radiopaque thermoresponsive hydrogel formulation at 2 ml/min. Increasing needle gauge and catheter length increases force required for injection. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared to Cook needle 18 G 7 cm. Data shown are represented as the mean $\pm$ SEM ($n = 3$)

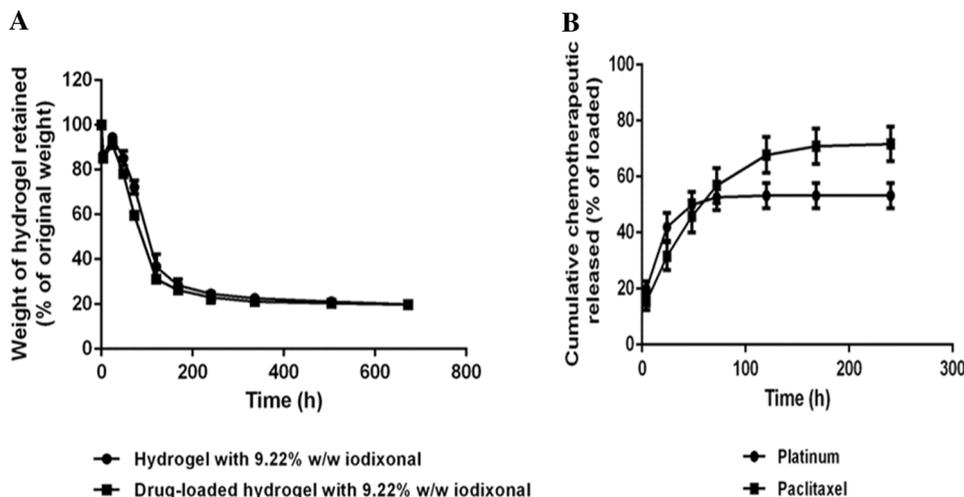**Fig. 5** **a** Disintegration of the blank and drug-loaded radiopaque thermoresponsive hydrogel over 28 days. Disintegration determined by measuring loss in mass (% of original weight of hydrogel) per timepoint. **b** Cisplatin (measured as platinum) and paclitaxel were released in a controlled manner from the drug-loaded radiopaque thermoresponsive hydrogel over a 10 day period. Cumulative percentage of loaded cisplatin and paclitaxel released over a 10-day period (240 h). Data shown are representative of the mean of three independent experiments $\pm$ SEM ($n = 3$)

while maintaining its inherent thermoresponsive properties. Furthermore, the contrast-labelled thermoresponsive hydrogel is clearly visualised on a variety of in-use medical imaging modalities. The viscosity properties of the thermoresponsive hydrogels at room temperature permits hand injection through off-the-shelf needles and catheters. The hydrogel demonstrates a slow disintegration profile in vitro when held at body temperature, with concomitant sustained drug release. It is important to note that in vitro experiments were performed in a non-biological environment, and in vivo investigations are required to determine the disintegration profile and clinical efficacy of the thermoresponsive hydrogel. Preliminary in vitro characteristics suggest this novel thermoresponsive hydrogel may be suitable for application in locoregional drug delivery to solid tumours under image guidance.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and Animal Rights This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent For this type of study, informed consent is not required.

Consent for Publication For this type of study consent for publication is not required.

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